

HUMAN SCAD-RELATED MOLECULES, SCRM-1 AND SCRM-2

TECHNICAL FIELD

5 This invention relates to nucleic acid and amino acid sequences of SCAD-related molecules and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative and immune disorders.

BACKGROUND OF THE INVENTION

10 The short-chain alcohol dehydrogenases (SCADs) are a diverse family of oxidoreductase enzymes. SCAD family members are involved in all aspects of cell biochemistry and physiology, including metabolism of sugar, synthesis or degradation of fatty acids, and synthesis or degradation of glucocorticoids, estrogens, androgens, and prostaglandins E₂ and F_{2α}. SCADs are found in bacteria, plants, invertebrates, and
15 vertebrates. Alignment of the different family members reveals large homologous regions and clustered similarities indicating sites of structural and functional importance. Some of these sites are associated with a type of coenzyme-binding domain, although similarity between family members can extend beyond this domain. Family members typically show only about 15% to 30% identity between enzyme pairs. Over one third of the conserved
20 residues are glycine residues, showing the importance of conformational and spatial restrictions. (Baker, M.E. (1995) *Biochem. J.* 309:1029-1030; and Jornvall, H. et al. (1995) *Biochemistry* 34:6003-6013.)

Members of the SCAD family differ in substrate specificity, tissue distribution, and subcellular location. For example, rat retinol dehydrogenase, which catalyzes the rate
25 limiting step in retinoic acid synthesis, is located in microsomes, while 2, 4-dienoyl-CoA reductase, which is involved in breakdown of unsaturated fatty acids, is located in mitochondria. Hep27, recently identified as a member of the SCAD family, is located in the nucleus. Interestingly, Hep27 is upregulated in growth-arrested human hepatoblastoma (HepG2) cells. Resumption of DNA synthesis in these cells results in a
30 down-regulation of the Hep27 protein. This suggests a role for SCAD family members in cell proliferation and cancer. (Gabrielli, F. et al. (1995) *Eur. J. Biochem.* 232, 473-477.)

SCAD involvement in fatty acid and steroid metabolism implicates members of the

SCAD family in a variety of disorders. Steroid dehydrogenases, such as the hydroxysteroid dehydrogenases, are involved in hypertension, fertility, and cancer. (Duax, W.L. and Ghosh, D. (1997) Steroids 62:95-100.) Retinoic acid, a regulator of differentiation and apoptosis, has been shown to down-regulate genes involved in cell proliferation and inflammation. (Chai, X. et al. (1995) J. Biol. Chem. 270:3900-3904.) Such differences in distribution and substrate specificity are presumably due to the unique segments contained within each family member. (Jornvall, supra.)

SCAD family members share two conserved structural motifs. One motif consists of a tyrosine and a lysine separated by any three amino acid residues. This motif is typically located at about residue 150 in a 250-residue dehydrogenase. The tyrosine and lysine residues are likely to be important in catalysis. Support for the importance of these two residues comes from mutagenesis studies with Drosophila alcohol dehydrogenase, human 15-hydroxyprostaglandin dehydrogenase, and human 11 β -hydroxysteroid and 17- β -hydroxysteroid dehydrogenases. (Baker, supra.)

The other motif shared by SCAD family members consists of an adenosine monophosphate (AMP)-binding domain. This motif is typically located near the N-terminus and consists of a hydrophobic pocket containing three glycine residues in a seven amino acid sequence. (Baker, supra.)

Variation in both the pentapeptide catalytic motif and the AMP-binding domain can exist between family members, though these changes do not seem to affect the activity of these proteins. For example, the tyrosine residue in the pentapeptide motif is replaced by a methionine in E. coli enoyl-acyl-carrier protein (EnvM), by serine in human 2,4-dienoyl-CoA reductase, and by valine in S. cerevisiae sporulation specific protein (SPX19). Some members of this group also have differences in the AMP-binding domain, including an insertion of two residues and poor conservation in one of the three glycine residues. (Baker, supra.)

The discovery of new SCAD-related molecules and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, treatment, and prevention of cell proliferative and immune disorders.

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SUMMARY OF THE INVENTION

The invention features substantially purified polypeptides, SCAD-related molecules, referred to collectively as "ScRM" and individually as "ScRM-1" and "ScRM-2." In one aspect, the invention provides a substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1,

and a fragment of SEQ ID NO:2.

The invention further provides a substantially purified variant having at least 90% amino acid identity to the amino acid sequences of SEQ ID NO:1 or SEQ ID NO:2, or to a fragment of either of these sequences. The invention also provides an isolated and purified polynucleotide
5 encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a
10 fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2, as well as an isolated and purified
15 polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, a
20 fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4. The invention further provides an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4, as well as an isolated and purified polynucleotide having a sequence which is
25 complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the
30 group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2, the method comprising the steps of: (a) culturing
35 the host cell containing an expression vector containing at least a fragment of a polynucleotide

encoding the polypeptide under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2 in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2, as well as a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing an immune disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2.

The invention also provides a method for treating or preventing a cell proliferative disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2.

The invention also provides a method for detecting a polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2 in a biological sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide sequence encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:2 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide in the biological sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

BRIEF DESCRIPTION OF THE FIGURES AND TABLE

Figures 1A, 1B, 1C, and 1D show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:3) of ScRM-1. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co. Ltd., San Bruno, CA).

5 Figures 2A, 2B, 2C, 2D, 2E, and 2F show the amino acid sequence (SEQ ID NO:2) and nucleic acid sequence (SEQ ID NO:4) of ScRM-2. The alignment was produced using MacDNASIS PRO™ software.

Figures 3A and 3B show the amino acid sequence alignment between ScRM-1 (Incyte Clone 1240869; SEQ ID NO:1) and human Hep27 (GI 1079566; SEQ ID NO:5), produced using
10 the multisequence alignment program of LASERGENE™ software (DNASTAR Inc. Madison WI).

Figures 4A, 4B, 4C, 4D, and 4E show the amino acid sequence alignment between ScRM-2 (Incyte Clone 2060002; SEQ ID NO:2) and C. elegans alcohol dehydrogenase/ribitol dehydrogenase (GI 2731377; SEQ ID NO:6), produced using the multisequence alignment
15 program of LASERGENE™ software.

Table 1 shows the programs used to identify and characterize ScRM, and provides relevant descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

20 Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

25 It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

30 Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are cited for the purpose of
35 describing and disclosing the cell lines, vectors, and methodologies which are reported in the

publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

5 DEFINITIONS

"ScRM," as used herein, refers to the amino acid sequences, or variant thereof, of substantially purified ScRM obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

- 10 The term "agonist," as used herein, refers to a molecule which, when bound to ScRM, increases or prolongs the duration of the effect of ScRM. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of ScRM.

- An "allelic variant," as this term is used herein, is an alternative form of the gene encoding ScRM. Allelic variants may result from at least one mutation in the nucleic acid sequence and
15 may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

- 20 "Altered" nucleic acid sequences encoding ScRM, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same as ScRM or a polypeptide with at least one functional characteristic of ScRM. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding ScRM, and
25 improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding ScRM. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent ScRM. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility,
30 hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of ScRM is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine
35 and glutamine; serine and threonine; and phenylalanine and tyrosine.

The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of ScRM which are preferably at least 5 to about 15 amino acids in length, most preferably at least 14 amino acids, and which retain some biological activity or immunological activity of ScRM. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

- 10 "Amplification," as used herein, relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (See, e.g., Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, pp.1-5.)

- 15 The term "antagonist," as it is used herein, refers to a molecule which, when bound to ScRM, decreases the amount or the duration of the effect of the biological or immunological activity of ScRM. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of ScRM.

- As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind ScRM polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

- 20 The term "antigenic determinant," as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

- The term "antisense," as used herein, refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence.
- 35 Antisense molecules may be produced by any method including synthesis or transcription. Once

introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

5 As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic ScRM, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

10 The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" binds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of
15 complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition
20 comprising a given amino acid sequence," as these terms are used herein, refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding ScRM or fragments of ScRM may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a
25 carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts, e.g., NaCl, detergents, e.g., sodium dodecyl sulfate (SDS), and other components, e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.

"Consensus sequence," as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using XL-PCR™ (The Perkin-Elmer Corp.,
30 Norwalk, CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW™ Fragment Assembly system (GCG, Madison, WI). Some sequences have been both extended and assembled to produce the consensus sequence.

As used herein, the term "correlates with expression of a polynucleotide" indicates that the
35 detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding

ScRM, by Northern analysis is indicative of the presence of nucleic acids encoding ScRM in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding ScRM.

A "deletion," as the term is used herein, refers to a change in the amino acid or nucleotide
5 sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative," as used herein, refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or
10 immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "similarity," as used herein, refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word
15 "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization
20 probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target
25 sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent
30 identity can be determined electronically, e.g., by using the MegAlign™ program (DNASTAR, Inc., Madison WI). The MegAlign™ program can create alignments between two or more sequences according to different methods, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups.
35 The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is

calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity
5 between nucleic acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) *Methods Enzymol.* 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by varying hybridization conditions.

“Human artificial chromosomes” (HACs), as described herein, are linear
10 microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (See, e.g., Harrington, J.J. et al. (1997) *Nat Genet.* 15:345-355.)

The term “humanized antibody,” as used herein, refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more
15 closely resembles a human antibody, and still retains its original binding ability.

“Hybridization,” as the term is used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

As used herein, the term “hybridization complex” refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary
20 bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words “insertion” or “addition,” as used herein, refer to changes in an amino acid or
25 nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

“Immune response” can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which
30 may affect cellular and systemic defense systems.

The term “microarray,” as used herein, refers to an arrangement of distinct polynucleotides arrayed on a substrate, e.g., paper, nylon or any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

The terms “element” or “array element” as used herein in a microarray context, refer to
35 hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate," as it appears herein, refers to a change in the activity of ScRM. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of ScRM.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to a
5 nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which, comprise a region of unique polynucleotide sequence that specifically identifies
10 SEQ ID NO:3, SEQ ID NO:4, for example, as distinct from any other sequence in the same genome. For example, a fragment of SEQ ID NO:3, SEQ ID NO:4 is useful in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:3, SEQ ID NO:4 from related polynucleotide sequences. A fragment of SEQ ID NO:3, SEQ ID NO:4 is at least about 15-20 nucleotides in length. The precise length of the fragment of SEQ ID NO:3, SEQ
15 ID NO:4 and the region of SEQ ID NO:3, SEQ ID NO:4 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment. In some cases, a fragment, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

20 The terms "operably associated" or "operably linked," as used herein, refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the
25 sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or
30 microarray. As used herein, the term "oligonucleotide" is substantially equivalent to the terms "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility
35 to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and

stop transcript elongation, and may be pegylated to extend their lifespan in the cell. (See, e.g., Nielsen, P.E. et al. (1993) *Anticancer Drug Des.* 8:53-63.)

The term "sample," as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acids encoding ScRM, or fragments thereof, or ScRM itself, may
5 comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a solid support; a tissue; a tissue print; etc.

As used herein, the terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The
10 interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

As used herein, the term "stringent conditions" refers to conditions which permit
15 hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, e.g., formamide, temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the
20 hybridization temperature.

The term "substantially purified," as used herein, refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution," as used herein, refers to the replacement of one or more amino acids or
25 nucleotides by different amino acids or nucleotides, respectively.

"Transformation," as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the
30 insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of
35 the host chromosome, as well as transiently transformed cells which express the inserted DNA or

RNA for limited periods of time.

A "variant" of ScRM polypeptides, as used herein, refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, LASERGENE™ software.

The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to ScRM. This definition may also include, for example, "allelic" (as defined above), "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

THE INVENTION

The invention is based on the discovery of new human SCAD-related molecules (ScRM), the polynucleotides encoding ScRM, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative and immune disorders.

Nucleic acids encoding the ScRM-1 of the present invention were first identified in Incyte Clone 1240869 from the lung cDNA library (LUNGNOT03) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:3, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 1240869H1 (LUNGNOT03), 396293R1 and 396293F1 (PITUNOT02), 1382578F1 (BRAITUT08), and 1806716F6 (SINTNOT13).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Figures 1A, 1B, 1C, and 1D. ScRM-1 is 278 amino acids

in length and has three potential casein kinase II phosphorylation sites at residues T25, S125, and S232; eight potential protein kinase C phosphorylation sites at residues S16, S21, T25, T31, S62, S63, S232, and T236; and a short-chain alcohol dehydrogenase family signature sequence from residue S169 through A197. BLOCKS analysis identifies short-chain alcohol dehydrogenase motifs from residues K33 through G45, G108 through A118, G162 through E199, and N204 through G213. PRINTS analysis identifies glucose/ribitol dehydrogenase motifs from residues V34 through R51, G108 through V119, M156 through A172, Y182 through A201, R203 through S220, and R238 through E258; and short-chain alcohol dehydrogenase motifs from residues G108 through V119, and Y182 through A201. ProfileScan identifies a short-chain alcohol dehydrogenase family signature from residue G162 through G213. PFAM analysis identifies significant sequence identity with short-chain alcohol dehydrogenases. ScRM-1 contains a modified AMP-binding domain and a canonical catalytic site of short-chain alcohol dehydrogenases from residues T39 through G46 and Y182 through K186, respectively. As shown in Figures 3A and 3B, ScRM-1 has chemical and structural similarity with human Hep27 (GI 1079566; SEQ ID NO:5). In particular, ScRM-1 and human Hep27 share 56% identity, have almost identical molecular mass (29.9 kDa) and isoelectric points (9.0), and share a canonical SCAD catalytic domain. A region of unique sequence in ScRM-1 from about amino acid 222 to about amino acid 228 is encoded by a fragment of SEQ ID NO:3 from about nucleotide 694 to about nucleotide 714. Northern analysis shows the expression of this sequence in various libraries, at least 67% of which are proliferative and at least 34% of which involve immune response. Of particular note is the expression of ScRM-1 in reproductive and cardiovascular tissues.

Nucleic acids encoding the ScRM-2 of the present invention were first identified in Incyte Clone 2060002 from the ovarian cDNA library (OVARNOT03) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:4, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 2060002H1 (OVARNOT03), 1353231F1 (LATRTUT02), 996779R1 (KIDNTUT01), 949209R1 (PANCNOT05), 1275304F1 (TESTTUT02), 1308115R1 (COLNFET02), and 1004312R1 (BRSTNOT03).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:2, as shown in Figures 2A, 2B, 2C, 2D, 2E, and 2F. ScRM-2 is 564 amino acids in length and has nine potential casein kinase II phosphorylation sites at residues S21, T62, S208, S233, S249, T482, S507, S515, and S517; five potential protein kinase C phosphorylation sites at residues T103, T204, T354, T459, and T556; and a potential tyrosine kinase phosphorylation site at residue Y45. As shown in Figures 4A, 4B, 4C, 4D, and 4E, ScRM-

2 has chemical and structural similarity with C. elegans alcohol/ribitol dehydrogenase (GI 2731377; SEQ ID NO:6). In particular, ScRM and C. elegans alcohol/ribitol dehydrogenase share 35% identity, with identity highest over the novel N-terminal half of the C. elegans protein.

ScRM-2 and C. elegans similar to alcohol/ribitol dehydrogenase share two potential casein kinase

- 5 II phosphorylation sites and a potential protein kinase C phosphorylation site at residues S233 and S507, and T103 in ScRM-2, respectively. A region of unique sequence in ScRM-2 from about amino acid 115 to about amino acid 121 is encoded by a fragment of SEQ ID NO:4 from about nucleotide 361 to about nucleotide 381. Northern analysis shows the expression of this sequence in various libraries, at least 65% of which are proliferative and at least 24% of which involve
10 immune response. Of particular note is the expression of ScRM-2 in reproductive tissues.

The invention also encompasses ScRM variants. A preferred ScRM variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the ScRM amino acid sequence, and which contains at least one functional or structural characteristic of ScRM.

- 15 The invention also encompasses polynucleotides which encode ScRM. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising the sequence of SEQ ID NO:3. In a further embodiment, the invention encompasses the polynucleotide sequence comprising the sequence of SEQ ID NO:4.

The invention also encompasses a variant of a polynucleotide sequence encoding ScRM.

- 20 In particular, such a variant polynucleotide sequence will have at least about 70%, more preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding ScRM. A particular aspect of the invention encompasses a variant of SEQ ID NO:3 which has at least about 70%, more preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to SEQ ID NO:3. The
25 invention further encompasses a polynucleotide variant of SEQ ID NO:4 having at least about 70%, more preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to SEQ ID NO:4. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of ScRM.

- 30 It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding ScRM, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These
35 combinations are made in accordance with the standard triplet genetic code as applied to the

polynucleotide sequence of naturally occurring ScRM, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode ScRM and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring ScRM under
5 appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding ScRM possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the
10 nucleotide sequence encoding ScRM and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode ScRM and ScRM derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the
15 synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding ScRM or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID
20 NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:3, or a fragment of SEQ ID NO:4, under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less
25 than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about
30 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization
35 will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100

$\mu\text{g/ml}$ denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 $\mu\text{g/ml}$ ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

- 5 The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM
- 10 trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperature of at least about 25°C , more preferably of at least about 42°C , and most preferably of at least about 68°C . In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment,
- 15 wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

- Methods for DNA sequencing and analysis are well known in the art. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE® (Amersham Pharmacia Biotech Ltd., Uppsala, Sweden), Taq polymerase (The Perkin-Elmer Corp., Norwalk,
- 20 CT), thermostable T7 polymerase (Amersham Pharmacia Biotech Ltd., Uppsala, Sweden), or combinations of polymerases and proofreading exonucleases, such as those found in the ELONGASE™ amplification system (Life Technologies, Inc., Rockville, MD). Preferably, sequence preparation is automated with machines, e.g., the ABI CATALYST™ 800 (The Perkin-Elmer Corp., Norwalk, CT) or MICROLAB® 2200 (Hamilton Co., Reno, NV) systems, in
- 25 combination with thermal cyclers. Sequencing can also be automated, such as by ABI PRISM™ 373 or 377 systems (The Perkin-Elmer Corp., Norwalk, CT) or the MEGABACE™ 1000 capillary electrophoresis system (Molecular Dynamics, Inc., Sunnyvale, CA). Sequences can be analyzed using computer programs and algorithms well known in the art. (See, e.g., Ausubel, supra, unit 7.7; and Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, Inc, New York,
- 30 NY.)

- The nucleic acid sequences encoding ScRM may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence
- 35 from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic.

- 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) *Nucleic Acids Res.* 16:8186.) A third method, capture PCR, involves PCR
- 5 amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) *PCR Methods Applic.* 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g.,
- 10 Parker, J.D. et al. (1991) *Nucleic Acids Res.* 19:3055-306). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk genomic DNA (Clontech, Palo Alto, CA). This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO™ 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN) or another
- 15 appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

- When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T)
- 20 library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

- Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different
- 25 nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., Genotyper™ and Sequence Navigator™, (The Perkin-Elmer Corp., Norwalk, CT)), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially
- 30 preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

- In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode ScRM may be cloned in recombinant DNA molecules that direct expression of ScRM, or fragments or functional equivalents thereof, in appropriate host cells. Due to the
- 35 inherent degeneracy of the genetic code, other DNA sequences which encode substantially the

same or a functionally equivalent amino acid sequence may be produced and used to express ScRM.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter ScRM-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding ScRM may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223. and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.) Alternatively, ScRM itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (The Perkin-Elmer Corp., Norwalk, CT). Additionally, the amino acid sequence of ScRM, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman and Co., New York, NY.)

In order to express a biologically active ScRM, the nucleotide sequences encoding ScRM or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and polynucleotide sequences encoding ScRM. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding ScRM. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding ScRM and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no

additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic.

- 5 The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

- Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding ScRM and appropriate transcriptional and
10 translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, ch. 4, 8, and 16-17; and Ausubel, F.M. et al. (1995, and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch. 9, 13, and 16.)

- 15 A variety of expression vector/host systems may be utilized to contain and express sequences encoding ScRM. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g.,
20 cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

- In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding ScRM. For example,
25 routine cloning, subcloning, and propagation of polynucleotide sequences encoding ScRM can be achieved using a multifunctional E. coli vector such as Bluescript® (Stratagene) or pSport1™ plasmid (GIBCO BRL). Ligation of sequences encoding ScRM into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful
30 for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509.) When large quantities of ScRM are needed, e.g. for the production of antibodies, vectors which direct high level expression of ScRM may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

- 35 Yeast expression systems may be used for production of ScRM. A number of vectors

containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, supra; and
5 Grant et al. (1987) Methods Enzymol. 153:516-54; Scorer, C. A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of ScRM. Transcription of sequences encoding ScRM may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV. (Takamatsu, N. (1987)
10 EMBO J. 6:307-311.) Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., Hobbs, S. or Murry, L.E. in
15 McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding ScRM may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite
20 leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses ScRM in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

25 Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

For long term production of recombinant proteins in mammalian systems, stable
30 expression of ScRM in cell lines is preferred. For example, sequences encoding ScRM can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable
35 marker is to confer resistance to a selective agent, and its presence allows growth and recovery of

cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* or *apr* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; and Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14; and Murry, *supra*.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP) (Clontech, Palo Alto, CA), β glucuronidase and its substrate β -D-glucuronoside, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding ScRM is inserted within a marker gene sequence, transformed cells containing sequences encoding ScRM can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding ScRM under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding ScRM and that express ScRM may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of ScRM using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing

monoclonal antibodies reactive to two non-interfering epitopes on ScRM is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN, Section IV; Coligan, J. E. et al. (1997 and periodic supplements) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York, NY; and Maddox, D.E. et al. (1983) *J. Exp. Med.* 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding ScRM include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding ScRM, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Pharmacia & Upjohn (Kalamazoo, MI), Promega (Madison, WI), and U.S. Biochemical Corp. (Cleveland, OH). Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding ScRM may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode ScRM may be designed to contain signal sequences which direct secretion of ScRM through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid

sequences encoding ScRM may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric ScRM protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of ScRM activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the ScRM encoding sequence and the heterologous protein sequence, so that ScRM may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel, F. M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch 10. A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled ScRM may be achieved in vitro using the TNT™ rabbit reticulocyte lysate or wheat germ extract systems (Promega, Madison, WI). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, preferably ³⁵S-methionine.

Fragments of ScRM may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A Peptide Synthesizer (The Perkin-Elmer Corp., Norwalk, CT). Various fragments of ScRM may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Chemical and structural similarity exists between ScRM-1 and Hep27 from human (GI 1079566). In addition, ScRM-1 is expressed in tissues associated with cell proliferation and inflammation. Therefore, ScRM-1 appears to play a role in cell proliferative and immune disorders.

Chemical and structural similarity exists between ScRM-2 and alcohol/ribitol dehydrogenase from C. elegans (GI 2731377). In addition, ScRM-2 is expressed in tissues associated with cell proliferation and inflammation. Therefore, ScRM-2 appears to play a role in cell proliferative and immune disorders.

5 Therefore, in one embodiment, ScRM or a fragment or derivative thereof may be administered to a subject to treat or prevent a cell proliferative disorder. Such cell proliferative disorders can include, but are not limited to, actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers
10 including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

In another embodiment, a vector capable of expressing ScRM or a fragment or derivative
15 thereof may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified ScRM in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those provided
20 above.

In still another embodiment, an agonist which modulates the activity of ScRM may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those listed above.

In another embodiment, ScRM or a fragment or derivative thereof may be administered to
25 a subject to treat or prevent an immune disorder. Such immune disorders can include, but are not limited to, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema,
30 episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma,
35 Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis,

thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma.

In another embodiment, a vector capable of expressing ScRM or a fragment or derivative thereof may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified ScRM in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of ScRM may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those listed above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of ScRM may be produced using methods which are generally known in the art. In particular, purified ScRM may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind ScRM. Antibodies to ScRM may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of polyclonal antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with ScRM or with any fragment or oligopeptide thereof which has immunogenic properties. Rats and mice are preferred hosts for downstream applications involving monoclonal antibody production. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and

Corynebacterium parvum are especially preferable. (For review of methods for antibody production and analysis, see, e.g., Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.)

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to ScRM have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 14 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of ScRM amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to ScRM may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, R.J. et al. (1983) *Proc. Natl. Acad. Sci.* 80:2026-2030; and Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) *Proc. Natl. Acad. Sci.* 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda, S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce ScRM-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) *Proc. Natl. Acad. Sci.* 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) *Proc. Natl. Acad. Sci.* 86: 3833-3837; and Winter, G. et al. (1991) *Nature* 349:293-299.)

Antibody fragments which contain specific binding sites for ScRM may also be generated. For example, such fragments include, but are not limited to, F(ab')₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) *Science* 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity and minimal cross-reactivity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of
5 complex formation between ScRM and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering ScRM epitopes is preferred, but a competitive binding assay may also be employed. (Maddox, supra.)

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for ScRM. Affinity is expressed as an
10 association constant, K_a , which is defined as the molar concentration of ScRM-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple ScRM epitopes, represents the average affinity, or avidity, of the antibodies for ScRM. The K_a determined for a preparation of monoclonal
15 antibodies, which are monospecific for a particular ScRM epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the ScRM-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require
20 dissociation of ScRM, preferably in active form, from the antibody. (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington, D. C.; and Liddell, J. E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York, NY.)

The titre and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For
25 example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is preferred for use in procedures requiring precipitation of ScRM-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

30 In another embodiment of the invention, the polynucleotides encoding ScRM, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding ScRM may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding ScRM. Thus, complementary molecules
35 or fragments may be used to modulate ScRM activity, or to achieve regulation of gene function.

Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding ScRM.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding ScRM. (See, e.g., Sambrook, supra; and Ausubel, supra.)

Genes encoding ScRM can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding ScRM. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding ScRM. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding ScRM.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may

be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be
5 prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding ScRM. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as
10 T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase
15 linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally
20 suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al.
25 (1997) Nature Biotechnology 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a
30 pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of ScRM, antibodies to ScRM, and mimetics, agonists, antagonists, or inhibitors of ScRM. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical
35 carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions

may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl
5 cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the
10 compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating,
15 dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized
20 powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of
25 ScRM, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

30 For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

35 A therapeutically effective dose refers to that amount of active ingredient, for example

ScRM or fragments thereof, antibodies of ScRM, and agonists, antagonists or inhibitors of ScRM, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of therapeutic to toxic effects is the therapeutic index, and it can be expressed as the ED_{50}/LD_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind ScRM may be used for the diagnosis of disorders characterized by expression of ScRM, or in assays to monitor patients being treated with ScRM or agonists, antagonists, or inhibitors of ScRM. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for ScRM include methods which utilize the antibody and a label to detect ScRM in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known

in the art and may be used.

A variety of protocols for measuring ScRM, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of ScRM expression. Normal or standard values for ScRM expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to ScRM under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of ScRM expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding ScRM may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of ScRM may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of ScRM, and to monitor regulation of ScRM levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding ScRM or closely related molecules may be used to identify nucleic acid sequences which encode ScRM. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding ScRM, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the ScRM encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequences of SEQ ID NO:3, SEQ ID NO:4, or from genomic sequences including promoters, enhancers, and introns of the ScRM gene.

Means for producing specific hybridization probes for DNAs encoding ScRM include the cloning of polynucleotide sequences encoding ScRM or ScRM derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S , or by enzymatic labels,

such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding ScRM may be used for the diagnosis of a disorder associated with expression of ScRM. Examples of such a disorder include, but are not limited to, cell proliferative disorders such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and immune disorders such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma. The polynucleotide sequences encoding ScRM may be used in Southern or Northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from patients to detect altered ScRM expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding ScRM may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding ScRM may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding ScRM in the sample indicates the presence of the associated disorder. Such

assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of ScRM, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding ScRM, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding ScRM may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding ScRM, or a fragment of a polynucleotide complementary to the polynucleotide encoding ScRM, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of ScRM include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; and Duplaa, C. et al. (1993) Anal. Biochem. 229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or

colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously
5 and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl.
10 Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding ScRM may be used to generate hybridization probes useful in mapping the naturally occurring genomic
15 sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial PI constructions, or single chromosome cDNA libraries. (See, e.g., Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical
20 chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, R.A. (ed.) Molecular Biology and Biotechnology, VCH Publishers New York, NY, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene
25 encoding ScRM on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such
30 as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using
35 positional cloning or other gene discovery techniques. Once the disease or syndrome has been

crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, ScRM, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between ScRM and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with ScRM, or fragments thereof, and washed. Bound ScRM is then detected by methods well known in the art. Purified ScRM can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding ScRM specifically compete with a test compound for binding ScRM. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with ScRM.

In additional embodiments, the nucleotide sequences which encode ScRM may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. [Attorney Docket No. PF-0559 P], filed July 16, 1998, are hereby expressly incorporated by reference.

EXAMPLES

I. cDNA Library Construction

LUNGNOT03

The LUNGNOT03 cDNA library was constructed using RNA isolated from non-tumorous
5 lung tissue removed from a 79 year old Caucasian male. Pathology for the associated tumor revealed a grade four carcinoma with Hürthle cells that had metastasized from thyroid cancer. Patient history included a benign prostate neoplasm and atherosclerosis.

OVARNOT03

10 The OVARNOT03 cDNA library was constructed using RNA isolated from non-tumorous ovary tissue obtained from a 43 year old Caucasian female during fallopian tube and ovary removal. Pathology for the associated tumor tissue indicated grade two mucinous cystadenocarcinoma. Patient history included viral hepatitis, cerebrovascular disease, atherosclerosis and mitral valve disorder. Family history included atherosclerotic coronary artery
15 disease, pancreatic cancer, stress reaction, cerebrovascular disease, breast cancer, and uterine cancer.

LUNGNOT03 and OVARNOT03

The frozen tissue was homogenized and lysed using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury NJ). The lysate was centrifuged over a 5.7 M CsCl
20 cushion using a Beckman SW28 rotor in a Beckman L8-70M Ultracentrifuge (Beckman Instruments) for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted with phenol chloroform at either pH 8.0 (LUNGNOT03) or pH 4.0 (OVARNOT03), precipitated using sodium acetate and ethanol, resuspended in RNase-free water, and treated with DNase. The RNA was re-extracted with phenol chloroform and precipitated as before. Poly(A+) RNA was isolated
25 using the Qiagen Oligotex kit (QIAGEN Inc., Chatsworth CA).

Poly(A+) RNA was used for cDNA synthesis and library construction according to the recommended protocols in the SuperScript plasmid system (Cat. #18248-013, Life Technologies, Gaithersburg, MD). cDNAs were fractionated on a Sepharose CL4B column (Cat. #275105-01, Pharmacia Amersham Biotech, Piscataway, NJ) and those cDNAs exceeding 400 bp were ligated
30 into pSPORT1 (Life Technologies, Inc.) and subsequently transformed into DH5 α ™ competent cells (Cat. #18258-012, Life Technologies).

II. Isolation of cDNA Clones

Plasmid DNA was released from the cells and purified using the Miniprep Kit (Catalog #77468; Advanced Genetic Technologies Corporation, Gaithersburg MD). This kit consists of a 96-well block with reagents for 960 purifications. The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific Broth (Catalog #22711, LIFE TECHNOLOGIES™) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after the cultures were incubated for 24 hours, the cells were lysed with 60 μ l of lysis buffer; 3) centrifugation for 5 minutes at 2900 rpm using a Beckman GS-6R rotor was performed before the block contents were added to the primary filter plate; and 4) addition of isopropanol to TRIS buffer was not routinely performed. The DNA samples were stored at 4°C.

III. Sequencing and Analysis

The cDNAs were prepared for sequencing using either an ABI PRISM CATALYST 800 (Perkin-Elmer Applied Biosystems, Foster City, CA) or a MICROLAB 2200 (Hamilton Co., Reno, NV) sequencing preparation system in combination with Peltier PTC-200 thermal cyclers (MJ Research, Inc., Watertown, MA). The cDNAs were sequenced using the ABI PRISM 373 or 377 sequencing systems and ABI protocols, base calling software, and kits (Perkin-Elmer Applied Biosystems). Alternatively, solutions and dyes from Amersham Pharmacia Biotech, Ltd. were used in place of the ABI kits. In some cases, reading frames were determined using standard methods (Ausubel, supra). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example V.

The polynucleotide sequences derived from cDNA, extension, and shotgun sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 1 summarizes the software programs used, corresponding algorithms, references, and cutoff parameters used where applicable. The references cited in the third column of Table 1 are incorporated by reference herein. Sequence alignments were also analyzed and produced using MACDNASIS PRO software (Hitachi Software Engineering Co., Ltd. San Bruno, CA) and the multisequence alignment program of LASERGENE software (DNASTAR Inc, Madison WI).

The polynucleotide sequences were validated by removing vector, linker, and polyA tail sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS to acquire annotation, using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length

polynucleotide sequences using programs based on Phred, Phrap, and Consed. and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. This was followed by translation of the full length polynucleotide sequences to derive the corresponding full length amino acid sequences. These full length polynucleotide and amino acid sequences were subsequently analyzed by querying against databases such as the GenBank databases described above and SwissProt, BLOCKS, PRINTS, PFAM, and Prosite.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; and Ausubel, supra, ch. 4 and 16.)

Electronic northern analysis was produced using analogous computer techniques. These techniques apply BLAST to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ™ database (Incyte Pharmaceuticals). The sensitivity of the computer search was modified to determine the specificity of the match. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score encompasses both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match may have a possibility of a 1% to 2% error, in contrast, a product score of 70 indicates that the match will be exact. Similar molecules were identified by product scores between 15 and 40, although lower scores may identify related molecules.

Electronic northern analysis further involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease categories included cancer, inflammation/trauma, fetal, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was divided by the total number of libraries across all categories. The results above were reported as a percentage distribution.

V. Extension of ScRM Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:3 and SEQ ID NO:4 were produced by extension of an appropriate fragment of the full length molecule, using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate extension of an antisense polynucleotide, and the other was synthesized to initiate extension of a sense polynucleotide. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO™ 4.06 (National Biosciences, Plymouth, MN), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (GIBCO BRL) were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR™ kit (The Perkin-Elmer Corp., Norwalk, CT) and thoroughly mixing the enzyme and reaction mix. PCR was performed using the PTC-200 thermal cycler (MJ Research, Inc., Watertown, MA), beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, with the following parameters:

	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
	Step 3	68° C for 6 min
	Step 4	94° C for 15 sec
25	Step 5	65° C for 1 min
	Step 6	68° C for 7 min
	Step 7	Repeat steps 4 through 6 for an additional 15 cycles
	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
30	Step 10	68° C for 7:15 min
	Step 11	Repeat steps 8 through 10 for an additional 12 cycles
	Step 12	72° C for 8 min
	Step 13	4° C (and holding)

A 5 µl to 10 µl aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQUICK™ (QIAGEN Inc.), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μ l of ligation buffer, 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C. Competent *E. coli* cells (in 40 μ l of appropriate media) were transformed with 3 μ l of ligation mixture and cultured in 80 μ l of SOC medium. (See, e.g., Sambrook, *supra*, Appendix A, p. 2.) After incubation for one hour at 37°C, the *E. coli* mixture was plated on Luria Bertani (LB) agar (See, e.g., Sambrook, *supra*, Appendix A, p. 1) containing carbenicillin (2x carb). The following day, several colonies were randomly picked from each plate and cultured in 150 μ l of liquid LB/2x carb medium placed in an individual well of an appropriate commercially-available sterile 96-well microtiter plate. The following day, 5 μ l of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5 μ l from each sample was transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

	Step 1	94° C for 60 sec
	Step 2	94° C for 20 sec
	Step 3	55° C for 30 sec
	Step 4	72° C for 90 sec
20	Step 5	Repeat steps 2 through 4 for an additional 29 cycles
	Step 6	72° C for 180 sec
	Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the the nucleotide sequences of SEQ ID NO:3 and SEQ ID NO:4 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:3 and SEQ ID NO:4 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO™ 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μ Ci of [γ -³²P] adenosine triphosphate (Amersham, Chicago, IL), and T4

polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are substantially purified using a Sephadex™ G-25 superfine size exclusion dextran bead column (Pharmacia & Upjohn, Kalamazoo, MI). An aliquot containing 10⁷ counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA
5 digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN, Boston, MA).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature
10 under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots to film for several hours, hybridization patterns are compared visually.

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array
15 elements on the surface of a substrate. (See, e.g., Baldeschweiler, *supra*.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels
20 and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected
25 using software well known in the art such as LASERGENE™. Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena,
30 M. et al. (1995) Science 270:467-470; and Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

VIII. Complementary Polynucleotides

Sequences complementary to the ScRM-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring ScRM. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same
 5 procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO™ 4.06 software and the coding sequence of ScRM. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the ScRM-encoding transcript.

10 IX. Expression of ScRM

Expression and purification of ScRM is achieved using bacterial or virus-based expression systems. For expression of ScRM in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac*
 15 (*lac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express ScRM upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of ScRM in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis
 20 virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding ScRM by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases,
 25 or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, ScRM is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid,
 30 single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Pharmacia, Piscataway, NJ). Following purification, the GST moiety can be proteolytically cleaved from ScRM at specifically engineered sites. FLAG, an 8-amino acid

peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak, Rochester, NY). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN Inc, Chatsworth, CA). Methods for protein expression and purification are discussed in Ausubel, F. M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch 10, 16. Purified ScRM obtained by these methods can be used directly in the following activity assay.

X. Demonstration of ScRM Activity

ScRM activity is measured by the oxidation of NADPH to NADP in the presence of substrate. (Kunau and Dommes (1978) Eur. J. Biochem. 91:533-544.) ScRM is preincubated for 10 min. at 37 °C in 60 µM potassium phosphate (pH 7.4), 125 nM NADPH, and 0.2 µM CoASH. The reaction is started by addition of substrate (12.5 to 150 µM final concentration). Change in absorbance at 340 nm, due to the oxidation of NADPH to NADP, is measured using a spectrophotometer at 23 °C. Units of ScRM activity are expressed as µmoles of NADP formed per 15 minute. A reaction lacking ScRM is used as a control. ScRM may increase or decrease the level of NADPH oxidation, relative to the control, depending on the substrate used.

XI. Functional Assays

ScRM function is assessed by expressing the sequences encoding ScRM at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a 20 mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT™ (Life Technologies, Gaithersburg, MD) and pCR™ 3.1 (Invitrogen, Carlsbad, CA, both of which contain the cytomegalovirus promoter. 5-10 µg of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2 µg 25 of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP) (Clontech, Palo Alto, CA), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics- 30 based technique, is used to identify transfected cells expressing GFP or CD64-GFP, and to evaluate properties, for example, their apoptotic state. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide;

changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the
5 binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York, NY.

The influence of ScRM on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding ScRM and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved
10 regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success, NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding ScRM and other genes of interest can be analyzed by Northern analysis or microarray techniques.

15 XII. Production of ScRM Specific Antibodies

ScRM substantially purified using polyacrylamide gel electrophoresis (PAGE)(see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the ScRM amino acid sequence is analyzed using LASERGENE™
20 software (DNASTAR Inc.) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel supra, ch. 11.)

Typically, oligopeptides 15 residues in length are synthesized using an Applied
25 Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to KLH (Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity by, for example, binding the peptide to plastic, blocking with 1% BSA,
30 reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIII. Purification of Naturally Occurring ScRM Using Specific Antibodies

Naturally occurring or recombinant ScRM is substantially purified by immunoaffinity chromatography using antibodies specific for ScRM. An immunoaffinity column is constructed

by covalently coupling anti-ScRM antibody to an activated chromatographic resin, such as CNBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing ScRM are passed over the immunoaffinity column, and the column is
5 washed under conditions that allow the preferential absorbance of ScRM (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/ScRM binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and ScRM is collected.

XIV. Identification of Molecules Which Interact with ScRM

10 ScRM, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled ScRM, washed, and any wells with labeled ScRM complex are assayed. Data obtained using different concentrations of ScRM are used to calculate values for the number, affinity, and association of ScRM with the
15 candidate molecules.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited
20 to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table I

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACTL.FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%.
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value= 1.0E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fasta E value= 1.0E-8 or less Full Length sequences: fasta score= 100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS and PRINTS databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff, Nucl. Acid Res., 19:6563-72, 1991; J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Altwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424	Score= 1000 or greater; Ratio of Score/Strength = 0.75 or larger; and Probability value= 1.0E-3 or less
PFAM	A Hidden Markov Models-based application useful for protein family search.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Scores=10-50 bits, depending on individual protein families

Table 1 cont.

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Score= 4.0 or greater
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8: 186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. <i>supra</i> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	